Profiles of phenolic acids in apples during ripening and storage

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Introduction

Phenolic compounds constitute a substantial group of phenylpropanoids which are produced by plants as secondary metabolites with antioxidant properties. The spectrum and concentrations of phenolic acids serve as a convenient marker of plant resistance to biotic stresses. Phenolics form also an important component of animal diet. Apples are a widely available fruit exhibiting significant concentrations of antioxidants, especially phenolics and carotenoids. Generally, the concentrations and composition of antioxidants vary during ripening and subsequent handling of harvested fruits.

We report a study of phenolic acid contents and composition during ripening and storage of three apple cultivars originating from Station of Apple Breeding, IEB.



Material





Characterization of 3 apple cultivars during fruit development 3 months of storage: dry weight (%) **BONITA:** dry weight (%) KARNEVAL: dry weight (%) Sirius: dry weight (%) **GA** - gallic acid acid FerA - ferulic acid

Comparison of apples stored for 3 months under different conditions

Abbreviations:



(UEB I-406/1) is a hybrid between Topaz and Cripps Pink Plant Variety Rights EU 45354, granted 06.02.2017 Applied for US Plant Patent, 24.03.2017

Origin:	Institute of Experimental Botany Prague (Střížovice) CZ
Tree:	Diploid, medium vigorous, ramified, spreading, good branching with many fruiting spurs
Blossom:	Mid-season, flowers heavily
Picking time:	About 1 week after Golden Delicious
Productivity:	Precocious, high and mostly regular
Keeping quality:	In cool storage about six months
Fruit:	Size medium, shape globose with broad eye basin, stem thin and long, skin smooth russet free, green yellow ground color is covered on 80 - 100 % with pink to brightly red overcolor, flesh firm, crisp, juicy with good, slightly sour taste
Diseases:	Scab resistant based on Vf gen, low susceptibility to powdery mildew
Comment:	Late apple variety with very homogenous nicely red fruits outstanding an
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400



(UEB 3741/1) was produced by crossing Vanda with Cripps Pink (Pink Lady $^{\ensuremath{\mathbb{B}}}$) Community Plant Variety Rights EU 29620 from 18. 04. 2011

- Institute of Experimental Botany Prague (Strizovice), CZ Origin Diploid, medium vigorous, ramified, upright to spreading, good branching with
- many fruiting spurs Mid-season, flowers heavily and regularly, does not require fruit thinning
- Early October, about one week before Golden Delicious Picking time:
- Productivity: Precocious, heavy and regular
- Keeping quality: In natural storage until February
- Medium size, shape globose to conical, moderate ribbing, stem medium long, skin smooth, appears russet free, appearance outstanding, multi-coloured with prominent red stripes on yellow ground color, flesh white, juicy, finely acid with slightly aromatic good flavo
- scab (Vf) tolera



The changes in free phenolic acid contents in apples during ripening and harvest - from June to October (ug/g DW)

The effect of storage on free phenolic acid contents (ug/g DW)



Spectrum of free phenolic acids in apples in June, August and October - i.e. during harvest (%)

■ GA ■ ProA ■ VA ■ CaA ■ ChA ■ p-CuA ■ FerA ■ SiA





The highest content of free phenolic acids was found in non-matured fruits in June - then it declined towards October. Free phenolic acid contents were not decreased during storage

month

Chlorogenic acid represented practically 100% of free phenolic acids in apples during whole development of fruits and during storage (except harvested Bonita cvs.). The same results were obtained in peels and fleshes (not shown) of all cultivars.

Summary

- Phenolic acid concentrations as well as their spectrum in peels and fleshes

were dependent on the phase of fruit development - the highest level was present at the start of development. Glycoside-bound phenolic acid content increased later - in peels of harvested apples.

- Phenolic acid contents were preserved in stored apples. Slight effects of storage conditions were rather cultivar dependent.



Storage conditions:

Apples were harvested in October and stored for 3 months : 1) in **ULO** boxes under low oxygen (1,2%), CO2 (2,2%) and under the temperature 1C 2) in boxes **RT** - with regulated temperature only - under 1C 3) in storerooms SR - with the temperature fluctuated between 1 - 4C.

Spectrum of free phenolic acids in stored apples (%)

■ GA ■ ProA ■ VA ■ CaA ■ ChA ■ p-CuA ■ FerA ■ SiA



SiA - sinapic acid GeA - gentisic acid *p***-HBA** - *para*-hydroxybenzoic acid SyA - syringic acid CiA - cinnamic acid





Community Plant Variety Rights EU 20805 from 24, 08, 2007 United States patent PP 18,541 from 04, 03, 2008

- Institute of Experimental Botany Prague (Strizovice), CZ Origin:
- Triploid, vigorous, spreading, branching medium, fruiting spurs medium to long Tree: Mid-season, slightly before Golden Delicious, flower set medium, regular Blosson
- Towards mid-October, about 10 days after Golden Delicious, fruits hang mostly Picking time: sinaly without thinning

Productivity: Precocious, produces regular good crops

Keeping quality: In natural storage until April, eating maturity 4 weeks after picking Fruit: Medium to large, round, height : width ratio 0,92, stem long and medium thick some fine russet may be present in the stem cavity, ground color green yellow to yellow, occasionally with a slight reddish blush, flesh yellow, firm, crisp, fine grained, very juicy, well balanced sugar (14,7 % Brix) and acid level, rich flavour Resistant to scab (Vf), tolerant to powdery mildew, absence of bitter pit

The variety can be considered for organic production as well as for IFP Comment: systems, growing requirements seem to be similar to Jonagold except treatments against scab, nice appearance, very interesting variety with many good qualities Institute of Experimental Botany of the AS CR, v. v. i.

Sirius - peel - VI Sirius - peel -VIII Sirius-peel -X

- The major free phenolic acid in peels and fleshes of all cultivar was chlorogenic acid.

- The major glycoside-bound phenolic acid was protocatechuic acid (and caffeic acid in apple fleshes).

High content of glycoside-bound phenolic acids was found in non-matured fruits in June, then it decreased during July and August. The levels of glycoside-bound phenolic acids rise mainly in peels of harvested fruits. Protocatechuic and caffeic acids represented the major glycoside-bound phenolic acids during ripening and harvest, the ratio between them differed during fruit development. Protocatechuic acid was present predominantly in apple peels, caffeic acid predominated in fleshes.



The effect of storage on glycoside-bound phenolic acid contents

flesh

Karneval





The influence of storage conditions on concentrations of phenolic acids varied between

The major glycoside-bound phenolic acid was ProA in peels and CaA in fleshes (not shown): the similar ratio between all glycoside-bound phenolic acids (known from harvested apples) remained in all 3 variants of stored apples.

Methods Phenolic acid analysis

Sample preparation

Samples of approx. 50-200mg of fresh weight were homogenized in 80% (v/v) methanol in Eppendorf vial tubes using a mixer mill. After addition of isotopically labelled internal standards they were left in the fridge overnight. The mixture was then centrifuged and the solids were re-suspended in 80% methanol and extracted in ultrasonic bath. After centrifugation the combined supernatants were evaporated to water phase and acidified to pH 2. The acid solution was extracted three times by diethyl ether. This extract was prepared for free phenolic acid analysis.

450

200

150

100

50

Bonita peel 🖸 Bonita flesh

VIII

The acidified water phase was left in fume for approx. 30 min to remove rest of diethylether and then transferred into crimp vial. Isotopically labelled internal standards and concentrated HCI were added, crimped and heated at 105°C for 1 hour. After cooling the reaction mixture was transferred into falcon tube; pH was adjusted to value 2 and the mixture was three times extracted by diethyl ether. This extract was prepared for glycoside-bond phenolic acids analysis.

Spectrum of glycoside-bound phenolic acids in apples in June. August and October - i.e. during harvest (%)

■ GA ■ ProA ■ GeA ■ p-HBA ■ VA ■ CaA ■ ChA ■ SyA ■ p-CuA ■ FerA ■ SiA ■ CiA



Spectrum of glycoside-bound phenolic acids in stored apples (%)

Sirius

Sirius

GA ProA GeA p-HBA VA CaA ChA SyA p-CuA FerA SiA CiA Bonita - peel - SR Bonita - peel - RT Bonita - peel - ULO 3,4_ 0,9_ 0,7_____ 0,4 3,5 0,6 0,0 0,0 3,2 0,0 0,0 1.5 1.2

cultivars.

The diethyl ether extracts were evaporated by the rotation vacuum concentrator (RVC) and stored in a freezer box to the final analysis.

LC-MS analysis

The evaporated samples were dissolved in 0,2 ml of 50% methanol, transferred into 0.5 ml polypropylene vials and placed into cooled stack of autosampler. The partition of 5 µl was injected on LC-MS system consisting of autosampler with cooling stack, quaternary HPLC pump and triple-quad mass spectrometer equipped with electrospray interface.

The chromatographic analysis was performed using 50x2.1 mm HPLC column Kinetex C18 with ternary gradient water/acetonitrile/0.1% acetic acid.

The mass spectrometer was operated in the negative multiple SRM (single reaction monitoring) mode with acquisition 3 to 8 transition for each compound. The most abundant ion was used for quantification, the others for identity confirmation. The analytes were quantified by the multilevel calibration graph with deuterated compounds used as internal standards.

Acknowledgement: This work was supported by the Ministry of Education of the Czech Republic, project LTC 17034; within CA **COST Action CA 15136 - Eurocaroten.**



